SUMMARY AND CONCLUSION

8.1 Summary:

Chemotherapy induced nausea and vomiting (CINV) is one of the main side effects of cancer therapy. CINV result in serious metabolic disturbances, nutritional depletion and anorexia, deterioration of the patient's physical and mental status, esophageal damage and ultimately patient withdrawal from therapy. Almost all patients receiving chemotherapy experience CINV even after optimization of antiemetic treatments. Functional Living Index-Emesis (FLIE) is a matrix which assesses quality of life of cancer patients, suggest that CINV has intense negative effects on quality of life of patients. There is also a substantial financial burden associated with CINV due to the ever-increasing costs of antiemetic medications. Some of the examples include, intravenous palonosetron and Fosaprepitant costing $188.70 and $262.65 per dose respectively. One retrospective cohort study carried out in 19,139 patients calculated the mean costs of CINV visits, including inpatient, outpatient, and emergency room visits to be $5299 for the first chemotherapy cycle (a period up to 30 days) and mean per-patient CINV-associated costs to be $731. For some patients, the cost of managing CINV is greater than the cost of chemotherapy.

CINV occurs in two phases; acute phase and delayed phase. Acute CINV occurs within 1–2 h of chemotherapy administration and can last for up to 24 h while delayed CINV occurs more than 24 h after chemotherapy administration. The CINV process involves a complex interplay between neurotransmitters and receptors at various anatomical regions. The three main neuro transmitters and receptors involved in the regulation of nausea and vomiting are serotonin (5-HT) associated with 5-hydroxytryptamine (5-HT3) receptor, substance P (SP) associated with neurokinin-1 (NK-1) receptor, and dopamine associated with dopamine (D2) receptor. Because the onset of acute and delayed CINV often overlap after the initial day of chemotherapy, it remains a challenge to determine an appropriate antiemetic regimen, as patients may require alternate treatment regimens. The MASCC and ASCO guidelines recommend a 5-HT3 receptor antagonist plus dexamethasone for acute CINV and dexamethasone for delayed CINV among patients receiving multiple-day cisplatin. However, this regimen is marginally effective in controlling acute CINV and is less successful in controlling delayed CINV.

The treatment options which address both acute and delayed phases of emesis occurring during chemotherapy are very limited and less effective. The marketed formulations available are SUSTOL (Granisetron SC Injection) and SACUSO (Granisetron transdermal patch) for once a

weekly treatment. Currently no generic is available for both the drugs due to patent protection and proprietary polymer technology. However, these individual formulations still require combination with other antiemetics for effective treatment. Thus, additional research is necessary to optimize management strategies for multiple-day chemotherapy.

There is need of formulations which provide effective treatment and patient compliance. This can be achieved by designing long-acting dosage forms with combination of two drugs, which provide sustained drug release up to one week. Comparative *in-vivo* studies in human are required for approval of generic or branded product which are costly and time consuming. Prediction of pharmacokinetics in human from *in-vitro* and *in-silico* studies is facilitated by regulatory agencies through modelling and simulation approach to reduce cost and create platform for future research. Currently there is a need of *in-silico* pharmacokinetic models which can mechanistically link *in-vitro* and pharmacokinetic properties to predict *in-vivo* performance of antiemetic drugs.

Two drugs Amisulpride (acting on dopamine D2 receptor) and Granisetron (acting on 5-hydroxytryptamine 5-HT3 receptor) were chosen with objective that by acting on different receptors they can increase the efficacy against emetic episodes and prevent development of treatment tolerance. Clinical trials suggest that granisetron is more effective than other 5-HT3 antagonists in preventing delayed nausea and vomiting that occur more than 24 h after the first dose of chemotherapy. Amisulpride is an antagonist of dopamine D2 and D3 receptors, approved since the 1980s as treatment for psychosis, with a favourable safety profile, even when used at doses of 400– 800 mg/day. In a pilot study, a combination of ondansetron and a single 20 mg intravenous dose of amisulpride protected 83% of patients from vomiting and use of rescue medication in the acute phase following cisplatin chemotherapy. Amisulpride has also been shown to be effective at preventing post-operative nausea and vomiting.

A novel dual-drug loaded microspheres (also coined as Janus microspheres) of Amisulpride and Granisetron were prepared using double emulsion-solvent evaporation technique which release the drug over a period of once week and provide effective treatment for both acute and delayed emesis. Granisetron being water soluble drug was incorporated in the inner phase and amisulpride being water insoluble drug was incorporated along with oil/polymer-solvent phase. Most commonly used and efficient polymers such as Polylactic-co-glycolic acid (PLGA 50:50) and Polycaprolactone (PCL) were used for developing dual-drug loaded microspheres.

Preformulation studies were performed to confirm the identity of drug and excipients using melting point analysis, Fourier transformed infrared spectroscopy analysis and thermal analysis using differential scanning colorimetry. All the results were meeting the reported data.

Analytical methods using UV-visible spectrophotometer and High-performance liquid chromatography were developed for charactering drug loading and entrapment efficacy and *in-vitro* drug release form the formulations. These methods were validated for linearity, robustness, sensitivity, precision, accuracy and specificity. The developed method was found to be suitable for analysing the formulations.

Three types of *in-vitro* drug release methods were developed in phosphate buffer pH 7.4 to gain understanding of mechanism of drug release under different conditions. The Accelerated method (at temp of 50°C), the real time method (at temperature of 37°C) and gel diffusion method which mimic the bio relevancy. The accelerated method helped to understand drug release behaviour in short period of time and avoided waiting for results till real time analysis.

Data of drug release were fitted in zero order, first order, higuchi, Korsmeyer–Peppas, Hixon-Crowell and Weibull models to determine release kinetic pattern from Janus microspheres. The drug release of Amisulpride was best fitted with Korsmeyer-Peppas equation with R^2 close to 0.9. The release of Granisetron was best fitted to first order with R^2 close to 0.9. Weibull model fitting was performed for optimized formulations for *in-vivo* simulations.

Quality by design approach was chosen to develop and optimize the formulations. Quality target product profile (QTPP) and critical quality attributes (CQAs) were identified and risk assessment was performed. A definitive screening design was used to identify the most critical parameters affecting the formulation and process followed by response surface design which provided the best combination of parameters to achieve the desired QTPP.

From screening design, it was found that stirring speed was the most impacting parameter for particles size, the polymer concentrations were impacting the drug loading and entrapment efficiency of formulation. Based on the screening design outcome, the stirring speed of 800 rpm, stirring time of 8 hours, PLGA and PCL concentration of 250 mg and PVA concentration of 0.5% was finalized. Further, the ratio of drug: polymer and polymer: polymer was selected in the optimization of formulation using response surface design. It was observed that, drug: polymer ratio was impacting particle size and polymer: polymer ratio was impacting rug loading, entrapment efficiency and drug release from the formulations. Design space was established which can provide the optimized formulation parameters such as desired particle

size (D90) of 80–100-micron, maximum drug loading and entrapment efficiency of both drugs and desired drug release profile (achieving drug release of 40-50% at 4 hours in ACC method) which can provide desired *in-vivo* profile.

The optimized microspheres were uniform in size and shape. They were characterized in terms of FTIR analysis, thermal analysis, % yield, particle size, morphology, % drug loading, % encapsulation efficiency and *in-vitro* drug release testing. The data was satisfactory from CMC perspective of once a weekly sustained release formulation. The IVRT data was further used in the *in-silico* PBPK model to predict the *in-vivo* pharmacokinetics of developed formulations.

The optimized formulations were tested for physico-chemical stability at storage conditions of 2-8°C for 6 months and 25°C/60% RH for 3 months in stoppered and sealed 10 ml clear colourless tubular USP-type I glass vials. The stability study was performed to evaluate the impact of storage conditions on assay and particle size of formulations. The results indicate that formulations were stable up to 6 months at 2-8°C and 3 months at 25°C/60% RH. No significant changes were observed for the tested parameters.

The in-silico modelling was performed to develop model for prediction of *in-vivo* pharmacokinetics in humans from animal species. The allometric scaling predicted volume of distribution and clearance values for human from preclinical species. The predicted values were within 2-fold error from the observed data for finalized model. *In-vivo* pharmacokinetic profiles for human were predicted using Wajima and Dedrick approach. Out of the two approaches, Wajima approach was more suitable in predicting the human PK data from preclinical species.

The PBPK model was developed for Amisulpride and Granisetron using Gastroplus^{TM} version 9.8.2 from simulations plus. The developed model was validated using intravenous and extravascular data. The developed model was used further to predict *in-vivo* pharmacokinetics of optimized formulation after intramuscular/subcutaneous administrations. The model was tested for parameter sensitivity analysis which showed the impact of physiological parameters such as lipophilicity, fraction bound to plasma proteins and drug release models, which helped to validate the model further. The accuracy of PBPK model were validated by the fold error of each time point (FEi), average fold error (AFE) and absolute average fold error (AAFE) for each time point of the plasma concentration profile. The FEi values for all the time points were well within the limit of 0.3 to 3-fold error. The AFE and AAFE values were also found within the limit of 0.5 to 2-fold error.

The dose and target steady state levels were calculated based on pharmacokinetic data and dose ranging studies from literature. The target steady state levels were calculated from in-silico model built using Gastroplus after multiple dose simulation of individual drugs.

The dissolution models were used to predict the target drug release profile based on first order kinetics and using complex microsphere model in the DDDplus platform. The calculated theoretical release profiles helped in model building and providing the target drug release profile which the optimized formulations should achieve.

The *in-silico* predictions showed that, developed once a weekly formulation is able to provide sustained release profile up-to 5-6 days. This was further proved by running virtual bioequivalence studies between multiple dose formulation and optimized sustained release formulation. The virtual BE Study showed that Cmax and AUC levels were within 80-125% limits and proved the equivalency. Further, IVIVC was developed to establish safe space for bioequivalent formulations.

8.2 Conclusion:

Novel drug delivery system in the form of dual-drug loaded polymeric Janus microspheres have been prepared for treatment of CINV. The combination of both drugs acting on different receptors enhanced the efficacy of the treatment and patient compliance through once a weekly administration. Combined use of *in-vitro* and in-silico tools for design and development of formulations was utilized successfully. In conclusion, the present work meets the set objectives and the idea and concept can further be extended to other products with minimal use of *in-vivo* studies.